Transcriptomic Analyses Predict Enhanced Metabolic Activity and Therapeutic Potential of mTOR Inhibitors in Acne-Prone Skin

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Current acne therapies center on preventing new lesions in patients with acne. These therapies were historically found to be beneficial yet were chosen without knowledge of the specific changes in the skin that favor lesion development. A major challenge in developing new treatments is the incomplete understanding of nonlesional (NL), acne-prone skin's molecular characteristics. To address this, we compared RNA-sequencing data from NL skin of 49 patients with acne (denoted as NL acne [NLA]) with those from 19 healthy controls with no acne history. We found 77 differentially expressed genes in NLA (log fold change > 1; $P < .05$), including genes associated with innate immunity and epidermal barrier function. Notably, *KRT6C*, *KRT16*, *S100A8*, *S100A9*, and lactotransferrin were upregulated, and *LCE4A*, *LCE6A*, and *CTSE* were downregulated. Gene set enrichment analysis revealed that metabolic pathways were enriched in NLA skin, whereas keratinization was negatively enriched. To identify compounds that could shift the gene expression signature of NLA skin toward healthy control skin, we performed connectivity mapping with the Library of Integrated Network-Based Signatures. We identified 187 compounds, particularly mTOR inhibitors, that could potentially normalize the gene expression profile of acne-prone skin to that of healthy skin. Our findings indicate that NLA skin has distinct differences in epidermal differentiation, cellular metabolism, and innate immunity that may promote lesion formation and suggest that mTOR inhibitors could restore NLA skin toward a healthier state, potentially reversing the predisposition to lesion development.

Keywords: Acne, Metabolism, mTOR, Nonlesional, Transcriptomics

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INTRODUCTION

Acne vulgaris (acne) is a common, yet complex skin condition, that predominantly affects adolescents and young adults. The pathogenesis of acne remains only partially understood, with factors such as follicular hyperkeratinization, excess sebum production, colonization of the follicle by *Cutibacterium acnes*, and perifollicular inflammation contributing to its etiology (Gollnick, 2015). However, the molecular mechanisms underlying these processes, particularly in nonlesional (NL) skin, are less explored and could provide valuable insights into acne initiation and progression.

Many studies have underscored the significance of analyzing the skin's transcriptome to gain insights into dermatological conditions. Transcriptomic studies provide a comprehensive, unbiased overview of gene expression profiles that may contribute to disease phenotypes. In acne, analyzing the transcriptome of clinically normal NL skin could reveal predispositions to lesion formation and identify unique targets for therapeutic intervention. Although previous studies using gene expression microarrays did not find marked differences between NL skin from patients with acne and healthy controls (HCs), using more sensitive approaches, such as RNA sequencing (RNA-seq), with a larger sample size have the potential to unveil insights into the transcriptome of acne-prone skin (Kelhälä et al, 2014; Trivedi et al, 2006).

This study leverages existing RNA-seq data to compare the transcriptome of NL skin from individuals with acne (denoted as NL acne [NLA]) with those from the healthy skin of individuals with no history of acne (Hall et al, 2019[1]). By integrating differential gene expression analysis (DGEA), gene set enrichment analysis (GSEA), and transcription factor (TF) activity prediction, we identified alterations in biological pathways that are involved in epidermal differentiation, lipid metabolism, and innate immune responses in NLA skin. These findings not only highlight that NLA skin is distinct from healthy skin, but also suggest that such differences may prime the skin for lesion formation.

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Abbreviations: DGEA, differential gene expression analysis; GES, gene expression signature; GSEA, gene set enrichment analysis; HC, healthy control; LINCS, Library of Integrated Network-based Cellular Signatures; LTF, lactotransferrin; NL, nonlesional; NLA, nonlesional acne; Rapa, rapamycin; RNA-seq, RNA sequencing; TF, transcription factor

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Furthermore, by employing a connectivity mapping approach, we identify compounds that can normalize/restore the transcriptome of NLA skin. This exploratory study is crucial for developing additional therapeutics and may inform our understanding of individual susceptibility to acne, providing a foundation for more effective and perhaps preventative strategies for acne.

RESULTS

Study overview and sample demographics

We analyzed an existing RNA-seq dataset from both HC with no history of acne and NL skin of individuals with acne (NLA) to explore potential differences in gene expression (Hall et al, 2019[1]). The dataset consisted of full-thickness skin punch biopsy samples from the upper back of 19 HCs and 49 participants with NLA, including both males and females with acne (Figure 1 and Table 1). The NLA group had an average Investigator's Global Assessment score of 2, corresponding to mild-to-moderate acne severity. Age differences between the HC and NLA groups were not significant. Our comprehensive analysis encompassed DGEA, GSEA, TF activity prediction, and connectivity mapping (Figure 1). This thorough evaluation aimed to identify not only known acne-related factors such as hyperkeratinization, sebum production, and inflammation, but also to discover previously unknown associations through an unbiased approach. When performing the DGEA, we controlled for variables such as sequencing batch and sex to ensure that the observed gene expression differences were attributable to the tissue group (NLA or HC).

The transcriptome of patients with NLA differs from that of healthy individuals

Initially, we focused on global transcriptional differences in gene expression between HC and NLA skin by performing principal component analysis. This analysis revealed that HC and NLA skin distinctly separated along the first 2 principal components and clustered together by group, indicating global differences in the transcriptomes between these 2 groups (Figure 2). Next, we performed DGEA to identify changes in gene expression that may promote acne lesion formation. This analysis identified a total of 367 genes that are differentially expressed between HC and NLA (141 upregulated, 226 downregulated) (log fold change > 0, $P < .05$). Narrowing this differentially expressed gene list to include only those with an absolute log fold change >1, we found 77 genes that exhibited the most significant change: 24 genes were upregulated, and 53 were downregulated in NLA skin compared with those in HC skin (Figure 2) ($P < .05$).

Among the most significantly upregulated genes, we identified several with established roles in epidermal differentiation, innate immune response, and sebaceous gland biology—critical factors in acne pathogenesis. Notably, this group includes stress-related keratins *KRT6C* and *KRT16*, the calcium-binding proteins, *S100A8* and *S100A9*, and lactotransferrin (*LTF*). In addition, *MUC1*, a marker of differentiated sebocytes, was upregulated. Conversely, our findings revealed a significant downregulation of genes associated with epidermal differentiation, including late cornified envelope proteins *LCE4A* and *LCE6A*, along with cathepsin E (*CTSE*). Furthermore, extracellular matrix genes, such as *TNXB*, *PRG4*, *MFAP5*, and *MATN4*, were downregulated. The detailed list of differentially expressed genes is available (Supplementary Material). Our DGEA indicates that the transcriptome of NLA skin differs from that of HC skin, suggesting that gene alterations in NLA skin may contribute to subsequent lesion formation.

GSEA identifies elevated metabolic activity in NLA skin

To explore the biological processes that were significantly enriched in NLA skin, we performed GSEA with the Hallmark and Reactome gene sets (Gillespie et al, 2022; Liberzon et al, 2015). The analysis of the 50 Hallmark gene sets identified 17 that were positively enriched and 3 that were negatively enriched (adjusted $P < .01$) (Figure 3). Gene sets associated with metabolic pathways, such as oxidative phosphorylation, fatty acid metabolism, glycolysis, and cholesterol homeostasis, were among the most positively enriched gene sets, suggesting an elevated state of metabolic activity in NLA skin. In addition, positive enrichment was noted for the mTORC1 signaling pathway, which is pivotal in regulating cell metabolism, survival, and proliferation (Figure 3a and c). Positive enrichment was also observed for the IL-6—JAK—signal transducer and activator of transcription 3 (STAT3) signaling and inflammatory response gene sets, suggesting an enhanced immune activity in NLA skin. On the contrary, gene sets related to coagulation, UV response, and epithelial mesenchymal transition were negatively enriched.

GSEA using the Reactome gene sets provided additional granularity, enabling us to identify enrichment in skin-specific pathways. We found 23 Reactome gene sets that were positively enriched and 3 that were negatively enriched (adjusted $P < .01$) (Figure 3b). In examining the top positively enriched Reactome gene sets, we observed multiple metabolic and immune-related gene sets. This reinforces the idea of elevated metabolic and immune activity in NLA skin. Remarkably, the keratinization Reactome gene set was the most significantly negatively enriched (Figure 3b and c). This set contains genes involved in the terminal differentiation of keratinocytes, suggesting a decreased keratinocyte differentiation profile in NLA skin. Furthermore, the negative enrichment in elastic fiber formation and extracellular matrix organization suggests decreased properties of the of the dermal matrix or epidermal basement membrane in NLA skin.

SREBP TFs are predicted to be key upstream regulators in NLA skin

To identify the drivers of the transcriptomic differences between NLA and healthy skin, we estimated the activity of TFs using the decoupler package with TF regulons from CollecTRI (Badia-i-Mompel et al, 2022; Müller-Dott et al, 2023). A total of 10 TFs were identified as significantly active in NLA skin. Notably, SREBF1 (SREBP1) and SREBF2 (SREBP2) exhibited the highest activity scores (Figure 4). SREBP1 is predominantly involved in the regulation of genes for fatty acid synthesis, whereas SREBP2 regulates genes involved in cholesterol synthesis (Horton et al, 2003; Pai et al, 1998). The expression and activity of both SREPB1 and SREBP2 have been linked with sebocyte lipogenesis and acne (Harrison et al, 2007; Li et al, 2023; Rosignoli et al, 2003; Smith et al, 2008). However, SREBP activity and function have not been previously associated with NLA skin.

a

	n			Mean Age (SD)		Mean IGA (SD)	
	F	M	Total	F	M	F	M
Acne, Non-Lesional	20	29	49	26.75 (4.6)	24.07 (3.98)	2.15 (0.75)	2.07 (0.7)
Healthy Control	12	7	19	25.25 (4.25)	28.57 (3.05)	0 (0)	0 (0)

b

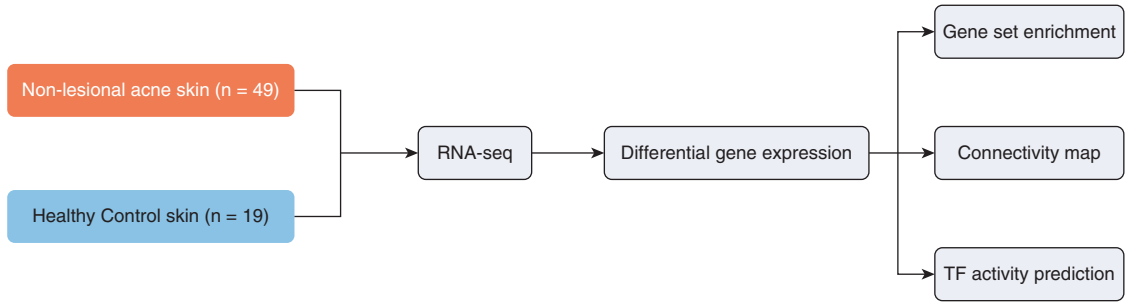

Figure 1. Summarized demographic information of study participants and schematic of study design. (**a**) Whole skin punch biopsies (4 mm) were collected from the upper back of 49 individuals with acne (NLA) and 19 controls with no history of acne (HC). Biopsies from the NLA group were collected from nonlesional skin that was at least 5 cm away from any active lesion. The table shows the number of male and female participants, mean age, and mean acne Investigator's Global Assessment score. **(b)** After RNA isolation from the biopsies, RNA-seq and subsequent analyses were performed. These included differential gene expression analysis, gene set enrichment analysis, TF activity prediction, and connectivity mapping to identify factors that distinguish NLA from HC skin and potential acne therapeutics. HC, healthy control; NLA, nonlesional acne; RNA-seq, RNA-sequencing; TF, transcription factor.

Connectivity mapping highlights mTOR inhibitors as candidates for acne intervention

The primary objective of acne therapy is to prevent the emergence of new lesions. To this end, we compared the gene expression signature (GES) of NLA skin with the chemical perturbagen signatures from the Library of Integrated Network-based Cellular Signatures (LINCS) (Subramanian et al, 2017). This was done to uncover potential therapeutic agents for acne. The GES for NLA skin comprised all differentially expressed genes (adjusted $P <$ .05). This GES was compared against chemical perturbation signatures within LINCS to identify compounds that could normalize the gene expression of NLA skin toward that of healthy skin. Our analysis identified 187 compounds with the potential to correct the abnormal gene expression of NLA skin (reversing compounds) (normalized connectivity score < -1) and 178 compounds that could replicate the gene expression profile of NLA skin (mimicking compounds) (normalized connectivity score $>$ 1) (Figure 5). Interestingly, among the reversing compounds, several are currently utilized or have been explored for acne treatment, such as tretinoin and calcipotriol (Mahran et al, 2024; Reynolds et al, 2024). In contrast, the mimicking compounds include compounds such as glucocorticoid and androgen receptor agonists, which induce acne (Arora et al, 2011).

Further enrichment analysis of the ranked compounds identified over-represented drug classes and targets. Notably, drug targets such as mTOR, PIK3CA, ATP1A1, and TUBB emerged as significantly over-represented among the reversing compounds. Conversely, the target NR3C1, a glucocorticoid receptor, was enriched in the mimicking compound group (Figure 5). This suggests a therapeutic potential for compounds targeting mTOR and PI3K signaling pathways in reversing the gene expression alterations of NLA skin. However, it also suggests caution with glucocorticoid receptor agonists because their effects on gene expression are similar to those observed in NLA skin.

To validate our connectivity mapping findings, we searched the National Center for Biotechnology Information Gene Expression Omnibus for public skin transcriptome datasets examining the effects of compounds or drug classes highlighted in Figure 5b and c. We identified 2 RNA-seq datasets: one evaluating the mTOR inhibitor rapamycin in mouse skin (GSE147950) and another evaluating the glucocorticoid receptor agonist clobetasol propionate in human skin (GSE120783) (additional details are provided in Materials and Methods). We compared the top Hallmark GSEA results from NLA skin with these datasets, representing reversing (rapamycin) and mimicking (clobetasol propionate) compounds. The comparison with clobetasol propionate revealed shared positively enriched gene sets, such as adipogenesis and fatty acid metabolism, showcasing that topical clobetasol can induce gene signatures similar to those of NLA skin. These findings are supported by the known adverse events associated with glucocorticoid use, including acneiform eruptions. In contrast, the NLA comparison with rapamycin identified several gene sets that were oppositely enriched, including oxidative phosphorylation, fatty acid metabolism, mTORC1 signaling, glycolysis, and cholesterol homeostasis, making it plausible that rapamycin may have similar effects in human skin. Epithelial–mesenchymal transition was negatively enriched in all comparisons (Figure 5f). Together, these comparative GSEA analyses support our computational approach to drug repurposing for identifying potential acne therapies. However, given the limited number of comparable datasets (each with their own limitations), experimental validation is essential in future studies.

Table 1. Patient Demographics

Sample ID	Group	Age, y	Sex	IGA
PT7001NL	Acne	29	Female	3
PT7002NL	Acne	25	Female	3
PT7003NL	Acne	18	Female	2
PT7004NL	Acne	18	Male	1
PT7005NL	Acne	21	Male	2
PT7006NL	Acne	20	Male	2
PT7007NL	Acne	30	Female	1
PT7008NL	Acne	30	Male	1
PT7009NL	Acne	26	Female	2
PT7011NL	Acne	29	Female	3
PT7012NL	Acne	31	Male	2
PT7013NL	Acne	30	Male	2
PT7014NL	Acne	29	Male	1
PT7016NL	Acne	18	Female	2
PT7017NL	Acne	31	Female	1
PT7018NL	Acne	24	Female	1
PT7019NL	Acne	34	Female	2
PT7020NL	Acne	32	Male	2
PT7021NL	Acne	31	Female	2
PT7022NL	Acne	32	Female	2
PT7023NL	Acne	26	Male	3
PT7024NL	Acne	25	Female	2
PT7025NL	Acne	27	Female	2
PT7026NL	Acne	25	Male	1
PT7027NL	Acne	28	Male	1
PT7029NL	Acne	19	Male	2
PT7030NL	Acne	21	Male	3
PT7031NL	Acne	29	Female	1
PT7032NL	Acne	22	Male	1
PT7033NL	Acne	22	Male	2
PT7034NL	Acne	25	Male	3
PT7035NL	Acne	20	Female	3
PT7036NL	Acne	23	Male	3
PT7037NL	Acne	26	Male	3
PT7038NL	Acne	24	Male	3
PT7039NL	Acne	19	Male	2
PT7040NL	Acne	25	Male	3
PT7041NL	Acne	30	Female	3
PT7043NL	Acne	22	Female	3
PT7044NL	Acne	20	Male	2
PT7045NL	Acne	21	Male	2
PT7046NL	Acne	30	Female	2
PT7047NL	Acne	30	Male	2
PT7048NL	Acne	23	Male	2
PT7050NL	Acne	22	Male	2
PT7051NL	Acne	22	Male	3
PT7053NL	Acne	22	Male	2
PT7054NL	Acne	25	Female	3
PT7056NL	Acne	22	Male	2
PT8002NL	Control	29	Female	0
PT8003NL	Control	29	Male	0
PT8004NL	Control	26	Male	0
PT8005NL	Control	26	Male	0
PT8006NL	Control	26	Male	0
PT8007NL	Control	31	Male	0
PT8008NL	Control	24	Female	0
PT8009NL	Control	24	Female	0
PT8010NL	Control	28	Male	0
PT8013NL	Control	23	Female	0
PT8014NL	Control	29	Female	0
PT8015NL	Control	25	Female	0
PT8016NL	Control	27	Female	0
PT8017NL	Control	20	Female	0
PT8018NL	Control	26	Female	0
PT8019NL	Control	34	Female	0
PT8021NL	Control	24	Female	0
PT8022NL	Control	18	Female	0
PT8023NL	Control	34	Male	0

Abbreviations: ID, identification; IGA, Investigator's Global Assessment.

DISCUSSION

In this study, we elucidated the transcriptomic differences between the individuals with NLA and HCs with no history of acne. By integrating DGEA, GSEA, and TF activity prediction, we uncovered pronounced differences in epidermal differentiation, cellular metabolism, and innate immune responses in NLA skin (Figure 6). These findings suggest that NLA skin possesses intrinsic molecular characteristics that may promote acne lesion formation. Furthermore, we applied connectivity mapping techniques to identify compounds that could potentially restore NLA skin to a healthier state.

Our findings indicate that even clinically normal-appearing NLA skin exhibits signs of epidermal stress, as evidenced by the significant upregulation of stress-related keratins such as KRT6C and KRT16, along with damage-associated molecular patterns such as S100A8 and S100A9. Typically, KRT6 and KRT16 expression is localized to glabrous skin and the outer root sheath of hair follicles, yet their expression increases in the interfollicular epidermis after injury or in inflammatory skin diseases (Lessard et al, 2013; Zhang et al, 2019; Zouboulis et al, 2020). A recent analysis of single-cell transcriptomic data from various skin conditions, including psoriasis and hidradenitis suppurativa, revealed that the presence of stress keratins such as *KRT6* and *KRT16* in lesional skin points to a shift in keratinocyte differentiation. This shift is associated with elevated expression of genes involved in innate immune function (Cohen et al, 2024). In line with this association, we observed a significant decrease in the enrichment of the Reactome keratinization gene set, which includes genes involved in keratinocyte differentiation and development of the epidermal barrier, suggesting that these processes are impaired in NLA skin. This impairment is similar to what has been previously observed in lesional acne skin, highlighting barrier dysfunction as a pathologic feature of acne (Dull et al, 2023). Our findings broaden this context to NL skin and suggest that impaired epidermal barrier function could predispose the skin to lesion formation. Although acne is not typically associated with overt epidermal barrier defects, functional studies of lesional acne skin have demonstrated increased transepidermal water loss and decreased levels of ceramides in the stratum corneum (Pappas et al, 2018; Yamamoto et al, 1995).

Given the link between acne and impaired epidermal barrier function, the upregulation of genes encoding

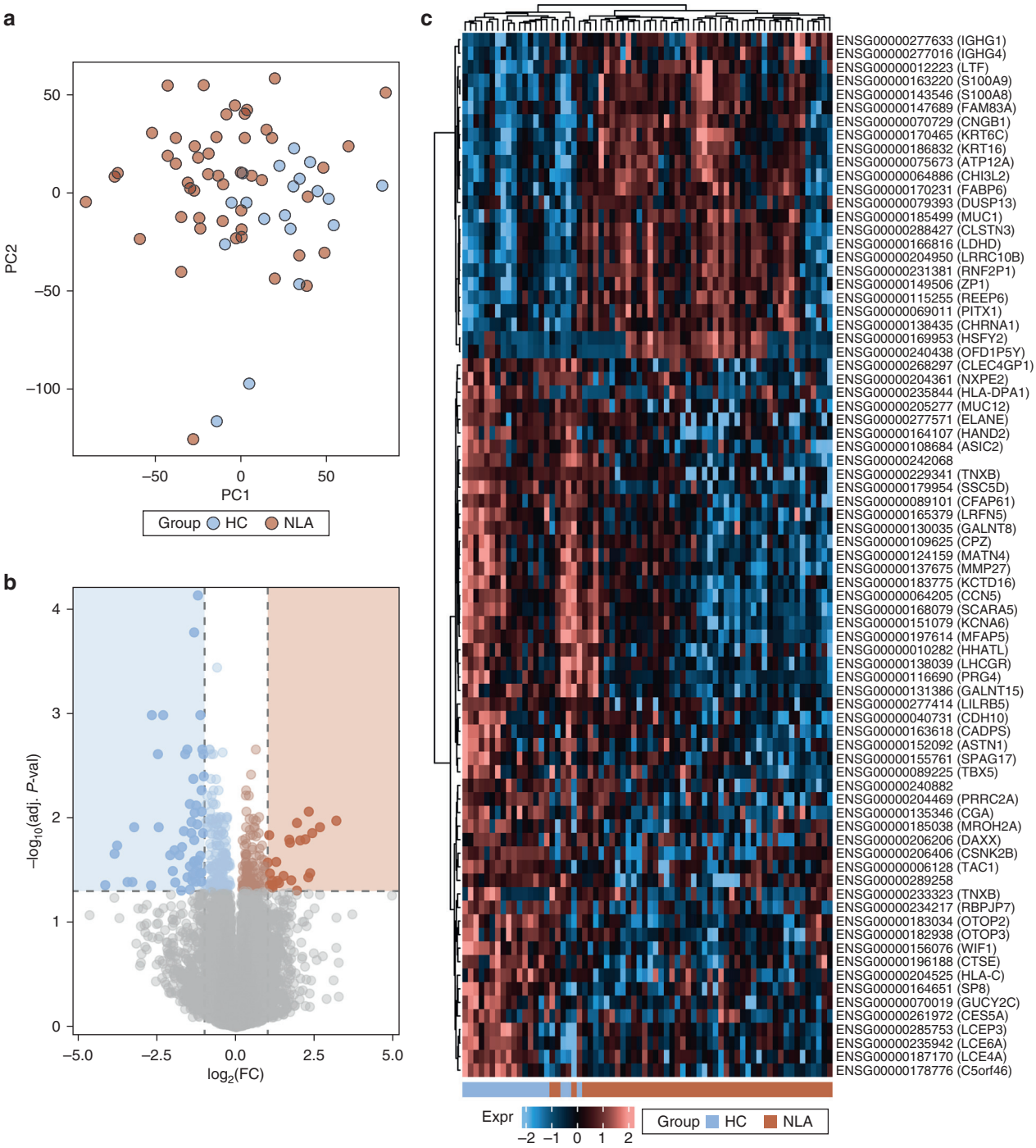

Figure 2. Differential gene expression analysis between individuals with acne (NLA) and HCs. (**a**) Plot showing the first 2 components from PC analysis colored by skin type: HC (blue) and NLA (red). **(b)** A volcano plot showing differentially expressed genes between NLA and HC, with the $\log_2$ FC on the x-axis and the $-\log_{10}$(adjusted *P*-value) on the y-axis. Red- and blue-shaded regions highlight genes that are the most significantly differentially expressed (absolute log FC > 1, adjusted $P < .05$). **(c)** Heatmap showing the Z-scored normalized counts for the most significantly differentially expressed genes (absolute log FC > 1, adjusted $P < .05$). Hierarchical clustering was performed on both the columns (patients) and rows (genes). Expr, expression level; HC, healthy control; log FC, log fold change; NLA, nonlesional acne; PC, principal component.

antimicrobial peptides such as the S100 proteins and lactotransferrin could reflect an activation of the innate immune response to skin commensals such as *C. acnes*. The proteins S100A8 and S100A9 form the calprotectin complex, which inhibits microbial activity by chelating manganese (II) ion and zinc (II) ion (Damo et al, 2013). Lactotransferrin, a multifunctional protein, not only sequesters ferric ion to prevent bacterial iron acquisition but also interacts with the bacterial cell surface through its affinity for negatively charged molecules, either causing membrane damage or inhibiting bacterial adhesion to host cells (González-Chávez et al, 2009). The involvement of these proteins in acne has

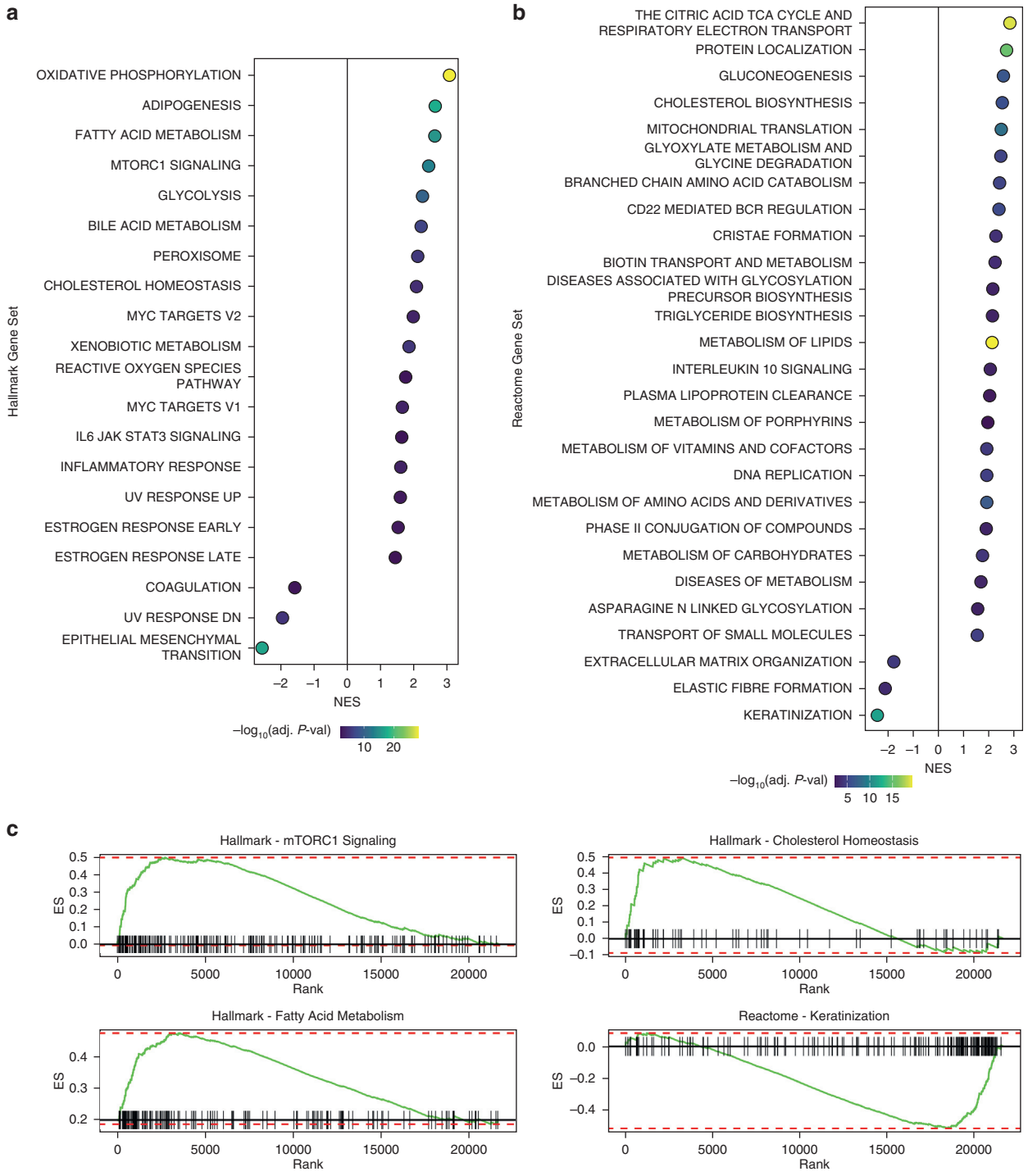

Figure 3. GSEA. (**a**) GSEA was performed using the Hallmark collection of gene sets from the molecular signatures database. Plot shows the significantly enriched (adjusted $P < .01$) gene sets ordered by their NES. Points are colored by $-\log_{10}$ (adjusted *P*-value). **(b)** Significantly enriched Reactome gene sets (adjusted $P < .01$). **(c)** Individual enrichment plots for mTORC1 signaling, fatty acid metabolism, cholesterol homeostasis, and keratinization gene sets. GSEA, gene set enrichment analysis; NES, normalized enrichment score.

been reported in previous studies. For instance, Fouda et al (2021) found higher serum calprotectin levels in patients with acne, correlating with acne severity. Elevated expression of S100A9 in acne lesions and increased lactotransferrin in follicular casts from acne skin relative to that in control skin have been reported (Bek-Thomsen et al, 2014; Kelhälä et al, 2014; Trivedi et al, 2006). S100A8/S100A9 proteins are expressed by immune cells and by keratinocytes in response to wounding or inflammation (Kerkhoff et al, 2012). Lactotransferrin is present in many body secretions, is an estrogen-responsive gene, and can be induced by innate triggers such as lipopolysaccharide or double-stranded RNA (Cao et al, 2022; Li et al, 2009). Although their precise role in acne is not well-understood, the upregulation of these molecules

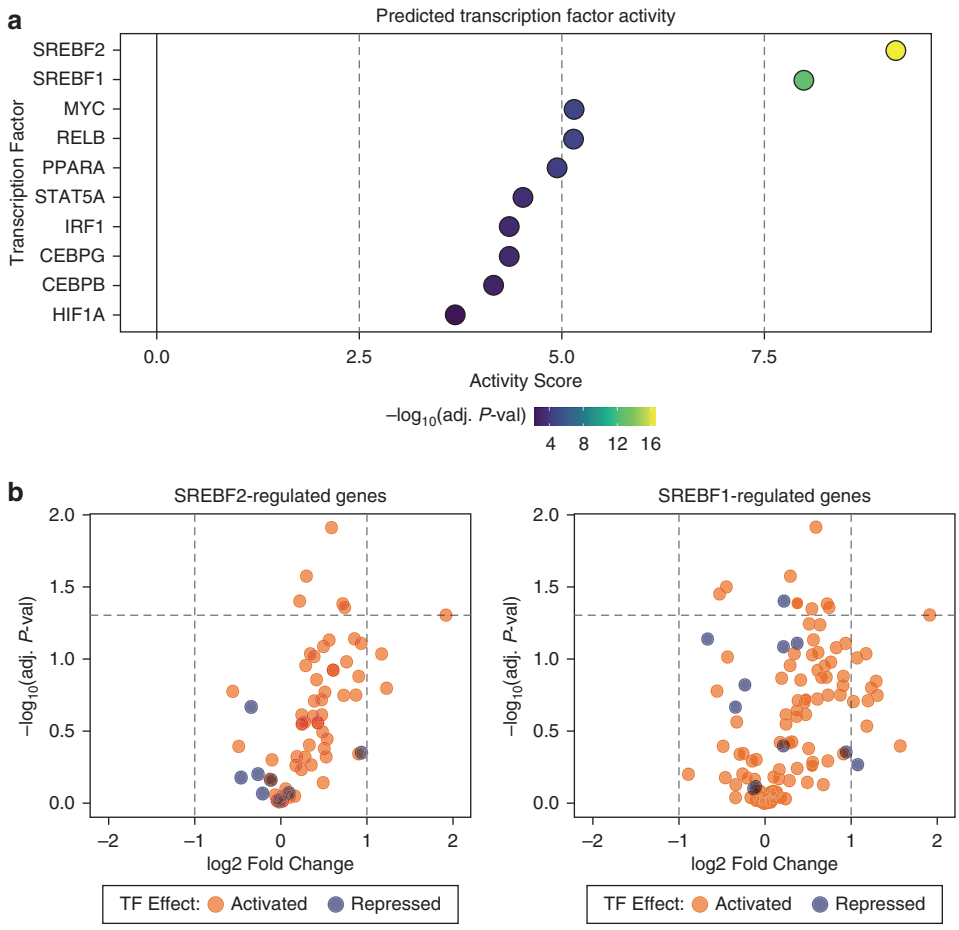

Figure 4. TF activity prediction suggests significant activation of SREBF1 and SREBF2 in NLA skin. (**a**) All TFs with predicted differential activity in NLA skin relative to those in healthy skin using the univariate linear model method from the decoupleR package (adjusted $P < .01$). **(b)** Volcano plots showing patterns of expression for SREBF2/SREBF1-regulated genes. Colors show the predicted effect of each TF–gene interaction from CollecTRI, with orange and blue indicating activation and repression of gene expression, respectively. NLA, nonlesional acne; TF, transcription factor.

supports a possible role in barrier disruption and innate immune activation in NLA skin.

The positive enrichment of gene sets related to lipid metabolism, glycolysis, and oxidative phosphorylation in our study suggests a compensatory mechanism by NLA skin to restore epidermal barrier properties in response to disrupted barrier processes. This aligns with the well-established link between compromised epidermal barrier function and the upregulation of lipid biosynthesis (Harris et al, 1997; Menon et al, 1985; Proksch et al, 1993). Specifically, the increased synthesis of ceramides, cholesterol, and fatty acids, which are essential components of the epidermal barrier, serves as a reparative mechanism after barrier disruptions. The predicted increase in the activities of SREBP1 and SREBP2, key transcriptional regulators for the synthesis of fatty acid and cholesterol, respectively, lends further support to this hypothesis (Horton et al, 2003; Pai et al, 1998). In addition, the gene set enrichment in lipid metabolism and the predicted activity of SREBP1 might also contribute to increased sebum production, a critical factor in acne pathogenesis. SREBP1 is expressed in sebaceous glands and activates lipogenesis, in vitro, in the SEB-1 sebocyte cell line (Harrison et al, 2007; Smith et al, 2008, 2006). In a study by Smith et al (2008), treatment of SEB-1 sebocytes with IGF1 led to SREBP1 activation and lipogenesis through the PI3-kinase (PI3K)/Akt signaling pathway. The roles of SREBP1 and SREBP2 in lipid metabolism and their association with sebocyte lipogenesis highlight a crucial link between lipid metabolism dysregulation and acne, suggesting that targeting these TFs or their downstream pathways could offer unique therapeutic strategies.

We found that mTORC1 signaling, which bridges PI3K/Akt signaling and SREBP1-mediated lipogenesis, was positively enriched in NLA skin (Porstmann et al, 2008). The connection between mTORC1 signaling and acne pathogenesis has been proposed previously, but empirical evidence from patient tissue is limited (Melnik, 2018). Lembo et al (2020) found increased mTOR gene expression in lesional acne skin, and Monfrecola et al (2016) demonstrated an increase in immunofluorescence staining intensity for phospho-S6 kinase 1, a key mTORC1 target, in both NL and lesional skin from patients with acne compared with that from HCs. Our finding of mTORC1 signaling enrichment in NLA skin strengthens the hypothesis that mTORC1 plays a role in acne pathogenesis.

Our connectivity mapping analysis further underscores the role of increased mTOR activity in acne, revealing that mTOR inhibitors are over-represented among reversing compounds capable of restoring NLA skin to a healthier state. This indicates the potential of mTOR inhibitors as an innovative therapeutic strategy for acne, particularly in a topical formulation. For example, rapamycin, known for its selective inhibition of mTORC1, has been effectively used in topical treatment of facial angiofibromas in patients with tuberous sclerosis complex, demonstrating a strong safety profile with

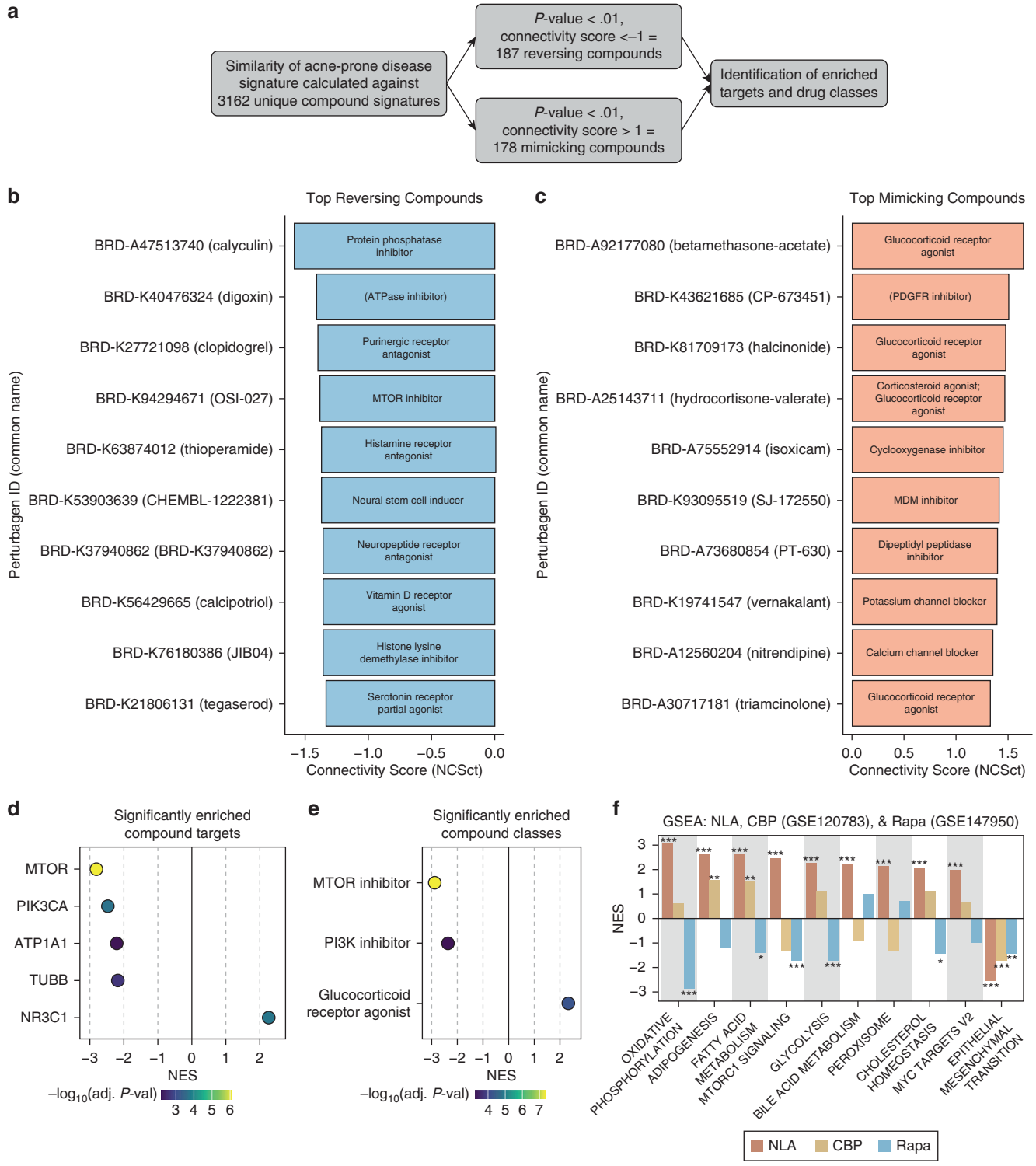

Figure 5. Connectivity mapping identified mTOR inhibitors as potential therapeutic candidates to reverse acne skin signature. (**a**) Workflow for compound prioritization: the similarity of the acne-prone disease signature (genes differentially expressed between NLA and HC) and LINCS signatures for chemical perturbagens with an annotated mechanism of action was calculated. There were 187 compounds that could potentially shift NLA gene expression toward HC (reversing; adjusted $P < .01$, normalized connectivity score < -1) and 178 compounds that could potentially shift HC gene expression toward NLA (mimicking; adjusted $P < .01$, normalized connectivity score > 1). Additional enrichment analysis was performed to identify over-represented drug classes among reversing and mimicking compounds. **(b, c)** Plots showing the top 10 reversing and mimicking compounds. The y-axes show the unique perturbagen identifier with the common name in parentheses. Mechanisms of action are annotated on the plot. **(d, e)** Significantly enriched compound targets and drug classes (adjusted $P <$ .01). **(f)** Comparative Hallmark GSEA results for this study (NLA) alongside those from public skin datasets GSE147950 (Rapa treated vs control mice) and GSE120783 (CBP treated vs untreated human skin). These public datasets are examples of a top reversing compound (mTOR inhibitor: rapa) or mimicking (glucocorticoid receptor agonist: CBP) compound. GSEA was performed separately for each indicted comparison. $^*P < .05$, $^{**}P < .01$, and $^{***}P < .001$. CBP, clobetasol propionate; GSEA, gene set enrichment analysis; HC, healthy control; LINCS, Library of Integrated Network-based Cellular Signatures; NCSct, normalized connectivity score summarized across cell types; NES, normalized enrichment score; NLA, nonlesional acne; Rapa, rapamycin.

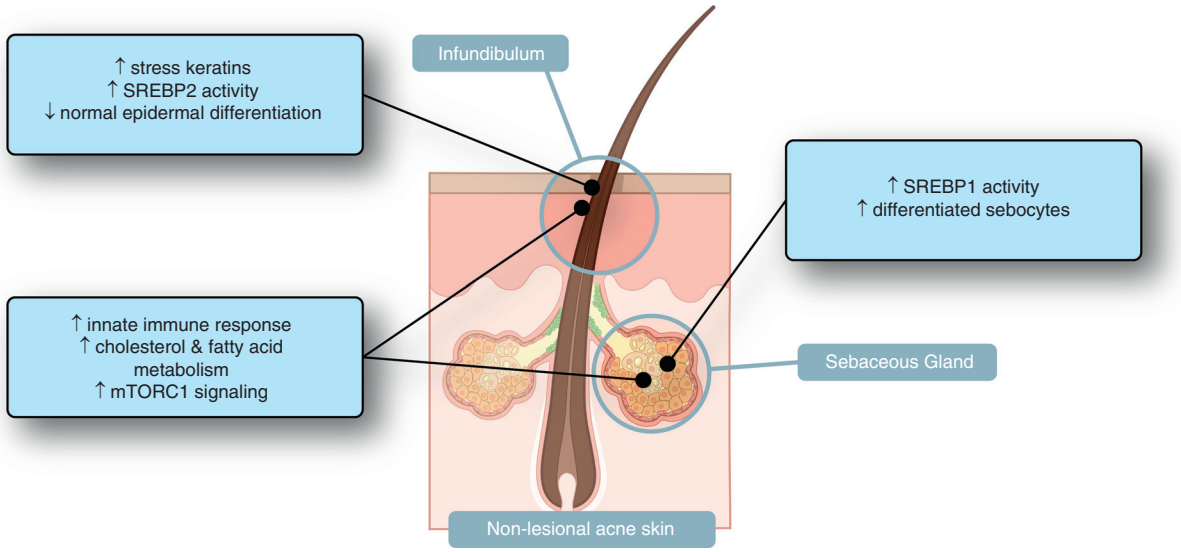

Figure 6. Diagram summarizing key findings and predicted follicular regions impacted.

negligible systemic absorption and minimal adverse effects (Aitken et al, 2023; Koenig et al, 2018). In addition, topical rapamycin has proven effective and well-tolerated in treating rosacea, which shares several pathological similarities with acne (Deng et al, 2021). In the study by Deng et al (2021), mTORC1 was shown to be activated in the lesional skin of patients with rosacea relative to that in HCs. Twice-daily application of topical rapamycin over 4 weeks resulted in significant clinical improvement in rosacea symptoms, underscoring the therapeutic promise of mTOR inhibition in skin conditions akin to acne.

Limitations

This study employed a comprehensive, unbiased computational approach to investigate the transcriptome of NLA skin and identify possible therapeutic targets. However, the absence of matched patient biopsies limited our ability to confirm the correlation between transcriptomic findings and protein expression in the skin. In addition, our connectivity mapping analysis was limited to LINCS compounds with known mechanisms of action, with the goal of identifying clinically relevant compounds. Exploring LINCS compounds with unknown mechanisms could broaden the spectrum of potential compounds but would require additional in vitro experiments or computational analysis to elucidate their effects. We used publicly available datasets for comparative GSEA analyses with NLA skin, recognizing the limitations, including different species, low sample number, and incomplete metadata, for an initial assessment to understand how identified compounds may impact acne skin. Importantly, experimental validation of the compounds identified by this drug repurposing analysis is necessary in future studies. Despite these challenges, analyzing data from nearly 70 HCs and patients with acne has unveiled previously unknown transcriptomic changes in acne-prone skin. This allows for the generation of hypotheses about key genes, pathways, and mechanisms involved in acne pathogenesis.

NL skin from individuals with acne shows significant transcriptomic differences from that of HCs, potentially making it more susceptible to developing acne lesions. Our analyses explored both established acne-related processes, such as hyperkeratinization, sebum production, and inflammation, and adopted an unbiased approach to uncover factors associated with acne onset. We discovered that in NLA skin, processes related to epidermal differentiation and cellular metabolism are altered in ways that potentially could affect the pilosebaceous unit, leading to acne lesions. Notably, our findings indicate an increase in mTORC1 signaling in NLA skin, suggesting that mTOR inhibitors could be promising agents for preventing lesion formation. Understanding these transcriptomic alterations, especially the potential role of mTOR inhibitors, opens new avenues for developing additional therapeutics to combat acne.

MATERIALS AND METHODS

RNA-seq data

Full-thickness human skin samples (4-mm punch biopsies) were obtained from the upper back of patients with mild-to-moderate acne and healthy volunteers in 2016 under an institutional review board–approved protocol (IF1719992; Mount Sinai School of Medicine) after written informed consent (Hall et al, 2019[1]). This previous study included 20 adults without a history of acne and 56 adults with mild-to-moderate acne. We performed a de novo RNA-seq analyses from NLA and HC skin using this original dataset (Hall et al, 2019[1]). The NLA biopsies were collected from areas at least 5-mm distant from any active acne lesion. Our de novo analysis included samples with sufficient reads from a single sequencing run to minimize batch effects, ultimately incorporating data from 49 NLA and 19 HC samples (Table 1).

Processing of RNA-seq data

Quality control and trimming of FASTQ files were performed using fastp (Chen et al., 2018). Read mapping and transcript quantification were performed using Salmon, with human genome and transcriptome files obtained from Ensembl (assembly GRCh38.14;

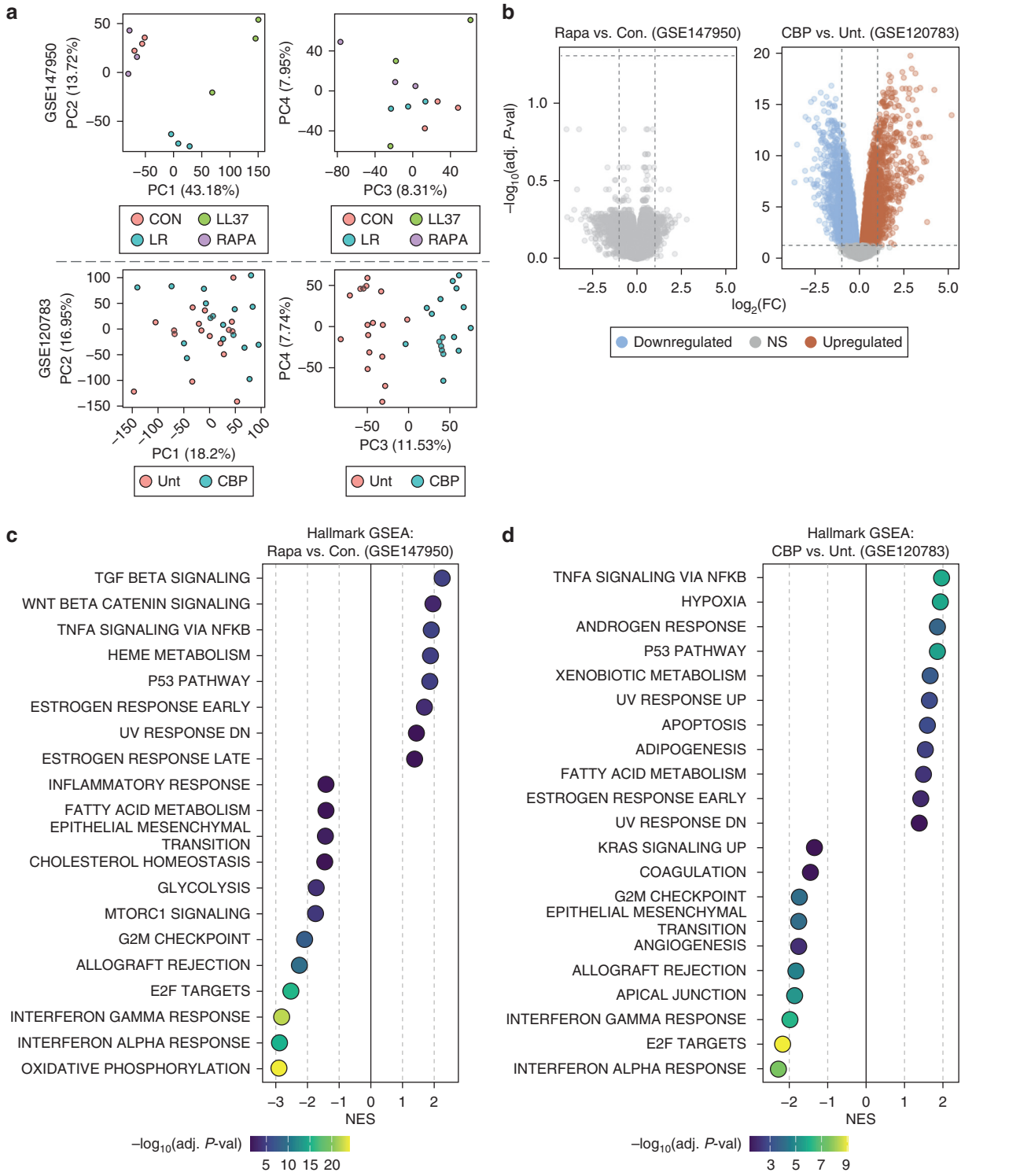

Figure 7. Transcriptomic analyses of public skin datasets GSE147950 and GSE120783. (**a**) PCA plots (PC1−PC4) color coded by groups. Validation groups for connectivity mapping include Rapa-treated and Con mice from GSE147950, along with human skin samples from topical CBP or Unt controls from GSE120783. **(b)** No genes were differentially expressed between Rapa and Con mouse skin (left volcano plot). A total of 7454 genes were differentially expressed between topical CBP and Unt skin (log_2 fold change > 0; adjusted $P < .05$). **(c, d)** GSEA using Hallmark gene sets from the molecular signatures database revealed 20 and 21 significantly enriched gene sets for the Rapa and Con and CBP and Unt comparisons, respectively (adjusted $P < .05$). CBP, clobetasol propionate treatment; Con, control; GSEA, gene set enrichment analysis; NS, not significant; PC, principal component; PCA, principal component analysis; Rapa, rapamycin; Unt, untreated.

release 109) (Martin et al, 2023; Patro et al, 2017). Gene-level counts were generated using the tximport package in R (Soneson et al, 2015).

DGEA and GSEA

We used the principal components analysis tools package for R to perform principal components analysis on the normalized count

matrix (Blighe and Lun, 2024). DGEA was conducted with edgeR and limma-voom (Law et al, 2014; McCarthy et al, 2012). Multiple comparisons adjustment of *P*-values was performed using the default Benjamini–Hochberg method in the Limma topTable function. The top differentially expressed genes (adjusted $P < .05$, absolute log fold change > 1) were used for heatmap generation using the ComplexHeatmap package (Gu, 2022). The list of differentially expressed genes, ranked by the moderated *t*-statistic, served as input for GSEA. GSEA was performed using the fGSEA package with the Hallmark and Reactome gene set collections sourced from the molecular signatures database (version 7.5.1) through the msigdbr package (Dolgalev, 2022; Gillespie et al, 2022; Korotkevich et al, 2021[2]; Liberzon et al, 2015; Subramanian et al, 2005). Gene sets with adjusted $P < .01$ were considered significantly enriched. We utilized the collapsePathways function from the FGSEA package to select independent Reactome pathways.

TF enrichment analyses

Estimation of TF activity was performed using the univariate linear model method from the decoupler package for R, with weighted TF regulons from CollecTRI (Badia-i-Mompel et al, 2022; Müller-Dott et al, 2023). We estimated aggregate differences in TF activity by running the univariate linear model on the list of genes output by the differential expression analysis ranked by the moderated *t*-statistic. We analyzed TFs with >20 annotated targets and used the Benjamini–Hochberg method for multiple comparisons adjustment. TFs with an adjusted $P < .01$ were considered significant.

Computational drug repurposing

Retrieval and processing of chemical perturbagen signatures. To discover compounds with potential therapeutic value for acne, we utilized the LINCS to perform connectivity mapping (Subramanian et al, 2017). We downloaded the level 5 chemical perturbation signatures and the associated metadata from the LINCS project from https://clue.io (Clue Data, 2020). Using the metadata, we filtered signatures to those that were from compounds with a known mechanism of action, were high quality (is_hiq = 1), and were exemplar (is_exemplar = 1) for a specific perturbagen and cell type. We limited our analysis to signatures from the 24-hour time point. In instances where multiple perturbagen doses for a given cell line were available, we retained the signature corresponding to the dose that induced the strongest transcriptional response, measured by the transcriptional activity score.

Constructing a query disease signature and connectivity mapping. To create a GES for NLA skin, we conducted a separate differential expression analysis of NLA versus healthy skin that was restricted to the Best INferred Genes gene space. Lists of upregulated and downregulated genes (adjusted $P < .05$) were used as input for the LINCS enrichment algorithm. We utilized the LINCS enrichment algorithm, as implemented in the signatureSearch R package, to calculate the similarity between our NLA acne skin signature and the perturbagen signatures (Duan et al., 2020). Compounds were considered significant if the weighted connectivity score had a false discovery rate <.01. Compounds with a negative weighted connectivity score (reversers) can shift the GES of NLA toward healthy, and compounds with a positive weighted connectivity score (mimickers) can shift the GES from healthy toward NLA.

[2] Korotkevich G, Sukhov V, Budin N, Shpak B, Artyomov MN, Sergushichev A. Fast gene set enrichment analysis. bioRxiv 2021.

Drug classes and target enrichment analysis. Further enrichment analysis was performed to identify drug classes and drug targets that were overrepresented among the compounds correlating with our disease signature. Drug sets and target sets were constructed by collapsing LINCS annotations according to their respective perturbagen_id. Compounds were ranked by their cell line collapsed enrichment score (normalized connectivity score summarized across cell type). The FGSEA package was utilized to compute the enrichment statistics. Drug classes and targets were considered with an adjusted $P < .01$.

This methodology enabled us to discern compounds that could potentially revert the transcriptional changes associated with NLA skin back to a state resembling HC skin, thereby identifying candidate molecules for further investigation as acne treatments.

Comparison with public datasets. To begin validation of our computational approach and predictions, we compared our data with publicly available datasets. We searched Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) for human or murine skin datasets (microarray or RNA-seq) that investigated any of the top 10 mimicking or reversing compounds or drug classes (Figure 5b and c). We identified 2 RNA-seq datasets—one evaluating the mTOR inhibitor rapamycin in mouse skin (GSE147950) and another evaluating the glucocorticoid receptor agonist clobetasol propionate in human skin (GSE120783)—and obtained the tables of raw gene counts from Gene Expression Omnibus. GSE147950 contained mouse skin data from Rapa-treated BALB/c mice (intraperitoneal injection; 4 mg/kg, 5 days; n = 3) and control mice (n = 3) (Deng et al, 2021, 2015). GSE120783 contained human skin data from a study where 0.05% topical clobetasol propionate was applied to the right arm of healthy subjects, and paired biopsies were collected from the right (treated) and left (untreated) arms after 24 hours (n = 17 subjects) (Sarkar et al, 2017). We performed differential expression analysis, accounting for the paired samples in GSE120783 and GSEA using the same methods as stated earlier (Figure 7). We then compared gene set enrichment results from these public data with those of the NLA skin to identify any pathways that were concordantly or discordantly enriched.

ETHICS STATEMENT

RNA-sequencing data were previously generated following institutional review board–approved protocols ((IF1719992; Mount Sinai School of Medicine)) for human subjects as described in Hall et al (2019[1]).

DATA AVAILABILITY STATEMENT

RNA-sequencing data analyzed in this article can be found at https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1114663, hosted at the Sequence Read Archive.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: MLS, AMN; Data Curation: MLS; Formal Analyses: MLS; Funding Acquisition: AMN; Investigation: MLS, GWA, DMT, AMN; Methodology: MLS; Project Administration: DMT, AMN; Resources: MLS, AMN; Software: MLS; Supervision: GWA, DMT, AMN; Validation: MLS, GWA, DMT, AMN; Visualization: MLS, AMN; Writing - Original Draft Preparation: MLS, AMN; Writing - Review and Editing: MLS, GWA, DMT, AMN

DECLARATION OF ARTIFICIAL INTELLIGENCE (AI) OR LARGE LANGUAGE MODELS (LLMS)

The author(s) did not use AI/LLM in any part of the research process and/or manuscript preparation.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at 10.1016/j.xjidi.2024.100306.

REFERENCES

Aitken P, Stanescu I, Boddington L, Mahon C, Fogarasi A, Liao YH, et al. A novel rapamycin cream formulation improves facial angiofibromas associated with tuberous sclerosis complex: a double-blind randomized placebo-controlled trial. Br J Dermatol 2023;189:520–30.

Arora MK, Yadav A, Saini V. Role of hormones in acne vulgaris. Clin Biochem 2011;44:1035–40.

Bek-Thomsen M, Lomholt HB, Scavenius C, Enghild JJ, Brüggemann H. Proteome analysis of human sebaceous follicle infundibula extracted from healthy and acne-affected skin. PLoS One 2014;9:e107908.

Blighe K, Lun A. PCAtools: everything principal components analysis. R package version 2.16.0. 2024. https://github.com/kevinblighe/PCAtools.

Cao X, Ren Y, Lu Q, Wang K, Wu Y, Wang Y, et al. Lactoferrin: a glycoprotein that plays an active role in human health. Front Nutr 2022;9:1018336.

Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 2018;34:i884–90.

Clue Data. Expanded CMap Lincs Resource 2020, https://clue.io; 2020. (accessed August 25, 2023).

Cohen E, Johnson CN, Wasikowski R, Billi AC, Tsoi LC, Kahlenberg JM, et al. Significance of stress keratin expression in normal and diseased epithelia. iScience 2024;27:108805.

Damo SM, Kehl-Fie TE, Sugitani N, Holt ME, Rathi S, Murphy WJ, et al. Molecular basis for manganese sequestration by calprotectin and roles in the innate immune response to invading bacterial pathogens. Proc Natl Acad Sci USA 2013;110:3841–6.

Deng Z, Chen M, Liu Y, Xu S, Ouyang Y, Shi W, et al. A positive feedback loop between mTORC1 and cathelicidin promotes skin inflammation in rosacea. EMBO Mol Med 2021;13:e13560.

Deng Z, Lei X, Zhang X, Zhang H, Liu S, Chen Q, et al. MTOR signaling promotes stem cell activation via counterbalancing BMP-mediated suppression during hair regeneration. J Mol Cell Biol 2015;7:62–72.

Dolgalev I. msigdbr: MSigDB gene sets for multiple organisms in a tidy data format, https://cran.r-project.org/web/packages/msigdbr/index.html; 2022 (accessed November 21, 2023).

Duan Y, Evans DS, Miller RA, Schork NJ, Cummings SR, Girke T. signatureSearch: environment for gene expression signature searching and functional interpretation. Nucleic Acids Res 2020;48:e124.

Dull K, Lénárt K, Dajnoki Z, Póliska S, Uchiyama E, Hendrik Z, et al. Barrier function-related genes and proteins have an altered expression in acne-involved skin. J Eur Acad Dermatol Venereol 2023;37:1415–25.

Fouda I, Obaid ZM, Hegazy SF, Samir Abd Al-Samie H, Nofal A. Calprotectin in acne vulgaris: a possible contributory role. J Cosmet Dermatol 2021;20:621–5.

Gillespie M, Jassal B, Stephan R, Milacic M, Rothfels K, Senff-Ribeiro A, et al. The reactome pathway knowledgebase 2022. Nucleic Acids Res 2022;50:D687–92.

Gollnick HP. From new findings in acne pathogenesis to new approaches in treatment. J Eur Acad Dermatol Venereol 2015;29:1–7.

González-Chávez SA, Arévalo-Gallegos S, Rascón-Cruz Q. Lactoferrin: structure, function and applications. Int J Antimicrob Agents 2009;33:301.e1–8.

Gu Z. Complex heatmap visualization. Imeta 2022;1:e43.

Harris IR, Farrell AM, Grunfeld C, Holleran WM, Elias PM, Feingold KR. Permeability barrier disruption coordinately regulates mRNA levels for key enzymes of cholesterol, fatty acid, and ceramide synthesis in the epidermis. J Invest Dermatol 1997;109:783–7.

Harrison WJ, Bull JJ, Seltmann H, Zouboulis CC, Philpott MP. Expression of lipogenic factors galectin-12, resistin, SREBP-1, and SCD in human sebaceous glands and cultured sebocytes. J Invest Dermatol 2007;127:1309–17.

Horton JD, Shah NA, Warrington JA, Anderson NN, Park SW, Brown MS, et al. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. Proc Natl Acad Sci USA 2003;100:12027–32.

Kelhälä HL, Palatsi R, Fyhrquist N, Lehtimäki S, Väyrynen JP, Kallioinen M, et al. IL-17/Th17 pathway is activated in acne lesions. PLoS One 2014;9:e105238.

Kerkhoff C, Voss A, Scholzen TE, Averill MM, Zänker KS, Bornfeldt KE. Novel insights into the role of S100A8/A9 in skin biology. Exp Dermatol 2012;21:822–6.

Koenig MK, Bell CS, Hebert AA, Roberson J, Samuels JA, Slopis JM, et al. Efficacy and safety of topical rapamycin in patients with facial angiofibromas secondary to tuberous sclerosis complex: the TREATMENT randomized clinical trial. JAMA Dermatol 2018;154:773–80.

Law CW, Chen Y, Shi W, Smyth GK. voom: precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol 2014;15:R29.

Lembo S, Di Caprio R, Balato A, Caiazzo G, Fabbrocini G, Skroza N, et al. The increase of mTOR expression is consistent with FoxO1 decrease at gene level in acne but not in psoriasis. Arch Dermatol Res 2020;312:77–80.

Lessard JC, Piña-Paz S, Rotty JD, Hickerson RP, Kaspar RL, Balmain A, et al. Keratin 16 regulates innate immunity in response to epidermal barrier breach. Proc Natl Acad Sci USA 2013;110:19537–42.

Li X, Zeng X, Kim D, Jiang J, Wei F, Zhang J, et al. Krüppel-like factor 4 (KLF4) facilitates lipid production in immortalized human sebocytes via regulating the expression of SREBP1. Biochem Biophys Res Commun 2023;667:146–52.

Li Y, Limmon GV, Imani F, Teng C. Induction of lactoferrin gene expression by innate immune stimuli in mouse mammary epithelial HC-11 cells. Biochimie 2009;91:58–67.

Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The molecular signatures database (MSigDB) hallmark gene set collection. Cell Syst 2015;1:417–25.

Mahran A, Ghazally A, Ali AS, Bakr RM. Efficacy and safety of calcipotriol as a potential topical treatment of acne vulgaris: a randomized, controlled, triple blinded, split-face clinical trial. Clin Exp Dermatol 2024;49:348–55.

Martin FJ, Amode MR, Aneja A, Austine-Orimoloye O, Azov AG, Barnes I, et al. Ensembl 2023. Nucleic Acids Res 2023;51:D933–41.

McCarthy DJ, Chen Y, Smyth GK. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. Nucleic Acids Res 2012;40:4288–97.

Melnik BC. Acne vulgaris: the metabolic syndrome of the pilosebaceous follicle. Clin Dermatol 2018;36:29–40.

Menon GK, Feingold KR, Moser AH, Brown BE, Elias PM. De novo sterologenesis in the skin. II. Regulation by cutaneous barrier requirements. J Lipid Res 1985;26:418–27.

Monfrecola G, Lembo S, Caiazzo G, De Vita V, Di Caprio R, Balato A, et al. Mechanistic target of rapamycin (mTOR) expression is increased in acne patients' skin. Exp Dermatol 2016;25:153–5.

Müller-Dott S, Tsirvouli E, Vazquez M, Ramirez Flores RO, Badia-I-Mompel P, Fallegger R, et al. Expanding the coverage of regulons from high-confidence prior knowledge for accurate estimation of transcription factor activities. Nucleic Acids Res 2023;51:10934–49.

Pai JT, Guryev O, Brown MS, Goldstein JL. Differential stimulation of cholesterol and unsaturated fatty acid biosynthesis in cells expressing individual nuclear sterol regulatory element-binding proteins. J Biol Chem 1998;273:26138–48.

Pappas A, Kendall AC, Brownbridge LC, Batchvarova N, Nicolaou A. Seasonal changes in epidermal ceramides are linked to impaired barrier function in acne patients. Exp Dermatol 2018;27:833–6.

Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. Salmon provides fast and bias-aware quantification of transcript expression. Nat Methods 2017;14:417–9.

Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, Leevers S, et al. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. Cell Metab 2008;8:224–36.

Proksch E, Holleran WM, Menon GK, Elias PM, Feingold KR. Barrier function regulates epidermal lipid and DNA synthesis. Br J Dermatol 1993;128:473–82.

Reynolds RV, Yeung H, Cheng CE, Cook-Bolden F, Desai SR, Druby KM, et al. Guidelines of care for the management of acne vulgaris. J Am Acad Dermatol 2024;90:1006.e1–30.

Rosignoli C, Nicolas JC, Jomard A, Michel S. Involvement of the SREBP pathway in the mode of action of androgens in sebaceous glands in vivo. Exp Dermatol 2003;12:480–9.

Sarkar MK, Kaplan N, Tsoi LC, Xing X, Liang Y, Swindell WR, et al. Endogenous glucocorticoid deficiency in psoriasis promotes inflammation and abnormal differentiation. J Invest Dermatol 2017;137:1474–83.

Smith TM, Cong Z, Gilliland KL, Clawson GA, Thiboutot DM. Insulin-like growth Factor-1 induces lipid production in human SEB-1 sebocytes via sterol response element-binding Protein-1. J Invest Dermatol 2006;126:1226–32.

Smith TM, Gilliland K, Clawson GA, Thiboutot D. IGF-1 induces SREBP-1 expression and lipogenesis in SEB-1 sebocytes via activation of the phosphoinositide 3-kinase/Akt pathway. J Invest Dermatol 2008;128:1286–93.

Soneson C, Love MI, Robinson MD. Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. F1000Res 2015;4:1521.

Subramanian A, Narayan R, Corsello SM, Peck DD, Natoli TE, Lu X, et al. A next generation connectivity map: L1000 platform and the first 1,000,000 profiles. Cell 2017;171:1437–52.e17.

Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA 2005;102:15545–50.

Trivedi NR, Gilliland KL, Zhao W, Liu W, Thiboutot DM. Gene array expression profiling in acne lesions reveals marked upregulation of genes involved in inflammation and matrix remodeling [published correction appears in J Invest Dermatol 2007;127:1825]. J Invest Dermatol 2006;126:1071–9.

Badia-I-Mompel P, Vélez Santiago J, Braunger J, Geiss C, Dimitrov D, Müller-Dott S, et al. decoupleR: ensemble of computational methods to infer biological activities from omics data. Bioinform Adv 2022;2:vbac016.

Yamamoto A, Takenouchi K, Ito M. Impaired water barrier function in acne vulgaris. Arch Dermatol Res 1995;287:214–8.

Zhang X, Yin M, Zhang LJ. Keratin 6, 16 and 17-critical barrier alarmin molecules in skin wounds and psoriasis. Cells 2019;8:807.

Zouboulis CC, Nogueira da Costa A, Makrantonaki E, Hou XX, Almansouri D, Dudley JT, et al. Alterations in innate immunity and epithelial cell differentiation are the molecular pillars of hidradenitis suppurativa. J Eur Acad Dermatol Venereol 2020;34:846–61.